An in vitro antifungal activity of various potencies of homeopathic medicines against Fusarium moniliforme plant fungus.

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Abstract: Fusarium moniliforme an emerging fungal pathogen has accounted affinity on dietary samples like corn, rice, sugarcane and vastly affects immunocompromised patients. Complementary and alternative medicines have precise action over this plant pathogen with increasing drug resistance. Fusarium moniliforme culture was procured from NCIM. Potato Dextrose Agar used as media for growth of culture at 28^0C (pH, 5.5) and kept in incubator for 48 hours. Optical density of suspension examined with spectrophotometer at 600nm. Homeopathic medicines Silicea, Mercurius Solubilis and Lachesis were used in various potencies for Agar well diffusion assay and Minimum inhibitory concentration (MIC) along with the positive control Bavistin (Carbendazim0.1mg/ml), Terbinafine (0.01mg/ml) and vehicle control of 90% ethyl alcohol individually. Homeopathic medicines Silicea 12C (13.5 ± 3.5) mm, Mercurius solubilis 6C (12.5 ± 5.5) mm, 12C (15 ± 2.0) mm and 1M (11.5 ± 1.5) mm; Lachesis 6C (15 ± 4.5) mm, 30C (17.5 ± 0.5) mm, 200C (15 ± 3.0) mm and 1M (16 ± 4.0) mm had inhibitory action against Fusarium moniliforme when performed by agar well diffusion assay. The MIC value of homeopathic medicines compared with vehicle control (ethanol 90%) and positive control Bavistin and Terbinafine had a significant difference in optical density value at 600nm. Homeopathic medicines Silicea (12C), Mercurius Solubilis (6C, 12C, 1M) and Lachesis (6C, 30C, 200C, 1M) showed maximum inhibition of growth against Fusarium moniliforme. Further studies are important in understanding the mechanism of action of these ultra-high diluted medicines.

Keywords– *Antifungal, Agar well diffusion, Fusarium moniliforme, Homeopathy, MIC.*

1. INTRODUCTION

Fusarium moniliforme, a fungal pathogen, has high affinity towards corn, rice, sugarcane and also vastly affects immunocompromised patients. [1,2,5,18] *F. moniliforme* (Sheld.) emend. Snyd & Hans has reported widespread occurrence of a *Neurospora* type of sporekiller phenomenon among natural populations in Ascomycetous plant pathogen with a worldwide distribution. [3] *F. moniliforme* produces the fumonisin mycotoxins B1 and B2 (FB1 and FB2) in maize, *Zea mays*. [4] *Fusarium* is now confirmed by several workers as a causal agent of Pokkah Boeng in sugarcane in Asia as well established pathogen by many workers. [5,6,7,8,9] Fusariocin A, a compound toxic to HeLa Cells is found in these *Fusarium* species. [10] *F. moniliforme* has toxicological effects confirmed in animal studies and is known to cause leukoencephalomalacia (LEM) in horses and has hepatocarcinogenic effects in rats and is highly toxic in variety of experimental animals. [11] *Fusarium* species are recognized as causes of keratomycosis and superficial burn wound infections. [12] Bavistin (carbendazim) is found commonly used in control of these Fusarium species but prolonged use of bavistin can lead to cytotoxic effects. [18] Complementary and alternative medicines have precise action over this plant pathogen in the ambit of increasing drug resistance. The antifungal effectiveness of various homeopathic medicines has been cited in various studies in recent times. [13,14]. Homeopathic medicines possessing drug as to vehicle ratio 1:99 is potentiated and denoted as centesimal scale (i.e. C) and further attenuation are prepared with same ratio from initial drug part. [15] In the present study homeopathic medicines namely *Silicea* [15], *Mercurius solubilis* [15] and *Lachesis* [15] of 6C, 12C, 30C, 200C, 1M, 10M, CM potencies were used in the research work (numbers indicate dilution of homeopathic remedy). These medicines are selected from Synthesis Repertory 9.0 [16] and Murphy Repertory [17] included under the rubric's used for treatment of fungal diseases in human studies. Therefore various potencies which are clinically used and available in the market for the above said medicines are used in this research work.

2. MATERIALS AND METHODS

2.1. Media and chemicals –

Media, reagents and chemicals used in this research work were procured from MERCK and Hi Media of AR grade. Bavistin 50% Wettable powder (carbendazim) [18] was purchased from the agrochemical shop and Terbinafine [19] was purchased from medical pharmacy.

2.2. Homeopathic medicine preparation –

Homeopathic medicines *Silicea, Mercurius solubilis* and *Lachesis* were obtained from GMP approved Manufacturer (St.George's) in hydro alcoholic liquid dilutions in 6C, 12C, 30C, 200C, 1M, 10M and CM potencies containing ethanol 90% as solvent base. Ethanol 90% was procured from Bharati Vidyapeeth Homeopathic Medical College, Department of Homeopathic Pharmacy, Pune; Maharashtra, India.

2.3. Micro-organism –

Plant fungus *Fusarium moniliforme* was procured from National Collection of Industrial Microorganisms (NCIM) Accession No. 1099 used as standard culture to examine antifungal activity.

2.4. Slide culture technique –

Morphological features of *Fusarium moniliforme* was confirmed by slide culture technique, adding one drop of Lacto-phenol cotton blue stain placed on glass slide with inoculated coverslip of culture. The preparation was examined using the low power (40X) objective of microscope. High-power (100X) objective in oil emersion was used to confirm observations. [13]

2.5. Agar well diffusion assay –

Agar wells were prepared with the help of sterilized borer within petri dish. *F.moniliforme* was inoculated into 10 mL of sterile potato dextrose broth and incubated at 28°C (pH, 5.5) for 48 hours. The culture used was 100µl spread on the surface of sterile potato dextrose agar plates using a spreader and then kept for incubation for 30 minutes, after which homeopathic medicines in their different potencies (20µl) were loaded in the wells containing culture. The plates were incubated at 28°C for 48 hours. [14] Experiments were carried out in duplicates and zone of inhibition measured in millimetres.

2.6. Minimum inhibitory concentration (MIC) –

MIC value of all medicines was found out using broth dilution method. Total volume in each tube was set to 1.5ml in the sequential order of potato dextrose broth; different potencies of homeopathic medicines and *F.moniliforme* respectively. The experiment was conducted with negative control (broth+culture), positive control (bavastin+broth+culture), (terbinafine+broth+culture) and vehicle control (Ethanol 90% + broth+ culture). Optical density of the *F. moniliforme* was measured after 24 hrs of incubation at 28°C (pH, 5.5) using Spectrophotometer at 600 nm. [14] Cultural inoculum was standardized for all experiment as OD 0.511A at 600nm. Experiments were carried out in duplicates.

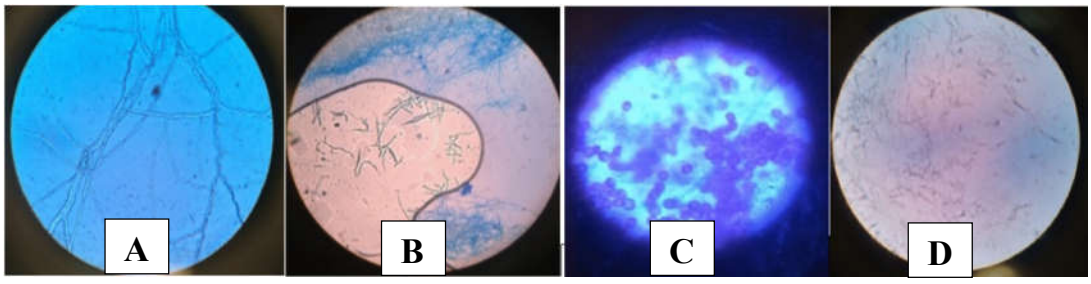
2.7. Statistical analysis –

The reading of experiments were calculated in mean value and standard error mean and analysed using Graph-Pad Prism version 7.0.(Graph-Pad Software, Inc. USA).

3. RESULTS

3.1. Slide culture technique results –

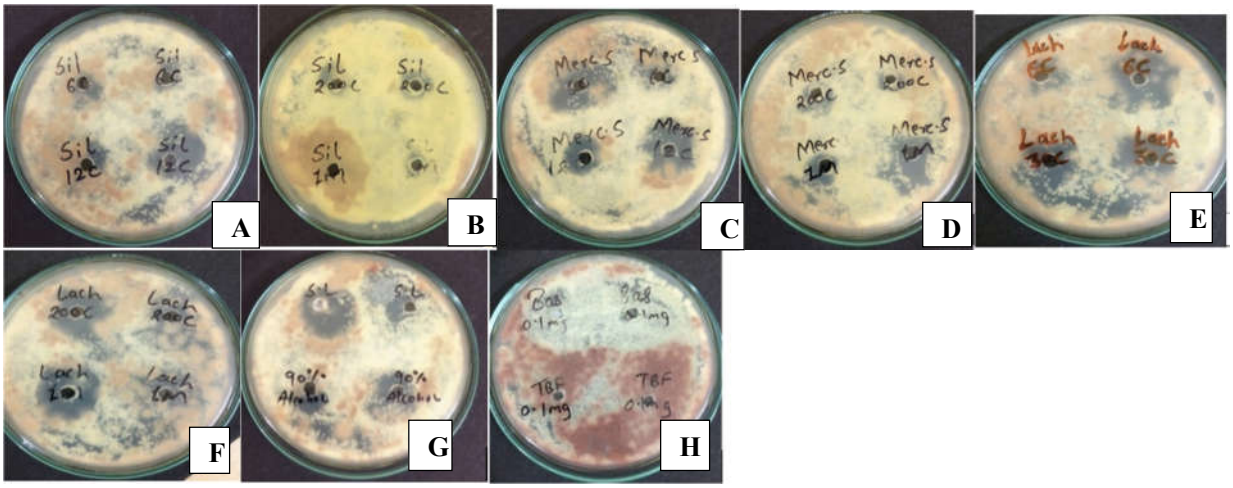
F. moniliforme were observed using slide culture technique under 40X and 100X objective. (Figure.1.).



[Figure.1 A &B) Visualization of Mycelium structure of *Fusariummoniliforme* at 40X. C & D) Visualization of Spore of *Fusariummoniliforme* at 100X.]

3.2. Agar Well Diffusion Assay results –

Homeopathic medicines *Silicea* (6C, 12C, 200C, 1M), *Mercurius solubilis* (6C, 12C, 200C, 1M) and *Lachesis* (6C, 30C, 200C, 1M) showed inhibition of zone ranged from 8 to 17.5 mm around the well (Table.1, Figure.2).



[Figure.2: A) *Silicea*- 6C, 12C. B) *Silicea*- 200C, 1M. C) *Mercurius solubilis*- 6C, 12C. D) *Mercurius solubilis*- 200C, 1M. E) *Lachesis* - 6C, 30C. F) *Lachesis* - 200C, 1M. G) Ethanol- 90%. H) Bavastin- 0.1mg/ml, Terbinafine- 0.01mg/ml.]

Table 1. Antifungal activity of Homeopathic Medicine.

Name of Medicine	Potency	Dilution Factor	Dosage (µl)	Mean Activity (in mm) ± SEM
Silicea	6C	10^{12}	20	8 ± 0
	12C	10^{24}	20	13.5 ±3.5
	30C	10^{60}	20	0
	200C	10^{400}	20	9 ± 1.0
	1M	10^{2000}	20	8.5 ± 0.5
	10M	10^{20000}	20	0
	CM	10^{200000}	20	0
Mercurius solubilis	6C	10^{12}	20	12.5 ± 5.5
	12C	10^{24}	20	15 ± 2.0
	30C	10^{60}	20	0
	200C	10^{400}	20	8 ± 1.0
	1M	10^{2000}	20	11.5 ± 1.5
	10M	10^{20000}	20	0
	CM	10^{200000}	20	0
Lachesis	6C	10^{12}	20	15 ± 4.5
	12C	10^{24}	20	0
	30C	10^{60}	20	17.5 ± 0.5
	200C	10^{400}	20	15 ± 3.0
	1M	10^{2000}	20	16 ± 4.0
	10M	10^{20000}	20	7±1.0
	CM	10^{200000}	20	0
Bavistin	0.1mg/ml		20	0
Terbinafine	0.01mg/ml		20	0
Ethanol	90%		20	8.5 ± 0.5

3.3. Minimum Inhibitory Concentration results –

The MIC of the drug and growth inhibition varied from 6C to CM potencies (Table.2).

Table 2. Minimum Inhibitory Concentration value of Homeopathic Medicine

Name of Medicine	Potency	Dilution Factor	Dosage (µl)	MIC Value (A) Mean ± SEM	MIC Value (A) (OD – 90% ethanol OD)	Inhibition Percentage
Silicea	6C	10^{12}	500	0.268 ± 0.06	-0.364	0%
	12C	10^{24}	500	0.151 ± 0.06	-0.481	0%
	30C	10^{60}	500	0.208 ± 0.04	-0.424	0%
	200C	10^{400}	500	0.244 ± 0.14	-0.388	0%
	1M	10^{2000}	500	0.145 ± 0.11	-0.487	0%
	10M	10^{20000}	500	0.230 ± 0.09	-0.402	0%
	CM	10^{200000}	500	0.247 ± 0.14	-0.385	0%
Mercurius Solubilis	6C	10^{12}	500	0.094 ± 0.05	-0.538	0%
	12C	10^{24}	500	0.219 ± 0.08	-0.413	0%
	30C	10^{60}	500	0.092 ± 0.01	-0.54	0%
	200C	10^{400}	500	0.201 ± 0.04	-0.431	0%
	1M	10^{2000}	500	0.164 ± 0.02	-0.468	0%
	10M	10^{20000}	500	0.147 ± 0.05	-0.485	0%
	CM	10^{200000}	500	0.143 ± 0.08	-0.489	0%
Lachesis	6C	10^{12}	500	0.316 ± 0.19	-0.316	0%
	12C	10^{24}	500	0.193 ± 0.11	-0.439	0%
	30C	10^{60}	500	0.098 ± 0.02	-0.534	0%
	200C	10^{400}	500	0.084 ± 0.02	-0.548	0%
	1M	10^{2000}	500	0.178 ± 0.01	-0.454	0%
	10M	10^{20000}	500	0.107 ± 0.05	-0.525	0%
	CM	10^{200000}	500	0.216 ± 0.08	-0.416	0%
Bavistin	0.1mg/ml		500	0.781 ± 0.21		
Terbinafine	0.01mg/ml		500	0.589± 0.02		
Ethanol	90%		500	0.632 ± 0.51		
Broth+Culture				0.637± 0.08		

4. DISCUSSION

The primary objective of study was to evaluate antifungal activity of homeopathic medicines. The medicines used in this study were selected from Synthesis 9.0 [16] and Murphy's Homeopathic Repertory [17]. From the results it is evident that selected medicines showed effective zone of inhibition in *F. moniliforme* in vitro, around the well in agar diffusion assay. Conversely, in positive control Bavistin (carbendazim0.1mg/ml) and Terbinafine (terbinafine hydrochloride at 0.01mg/ml) and vehicle control (ethanol 90%) the inhibitory effect was not significant. The results of MIC are interpreted in absorbance value of all potencies with percent growth inhibition, when compared to vehicle control (ethanol 90%); positive control Bavistin (Carbendazim0.1mg/ml) and Terbinafine (terbinafine hydrochloride at 0.01mg/ml) showed a significant difference in their optical density value at 600nm. The in-vitro study of Sachin Kumar Jain et al. (2014) Bavistin (carbendazim at 100ppm=0.01% i.e. 0.1mg/ml) by food poison technique showed cent percent inhibition towards *F.moniliforme* [18] and Mo´nica Azor et al. (2008) Terbinafine (terbinafine hydrochloride at 0.01mg=10µg/ml) by micro dilution method acted as most active drug against *F. verticillioides* (*Fusarium moniliforme*) [19] but in this research work both controls at respected concentration showed resistant to the culture *F. moniliforme*.

Homeopathic philosophy is always challenged with its ultra-high dilutions which are implausible to explain as they are beyond the Avogadro's constant unit ($6.023x10^{-23}$) [20], but in this research work the ultra-high dilution homeopathic medicines (10^{12} to 10^{200000}) has shown a significant result when compared with vehicle control and positive control. The results interpreted in this in-vitro study shows that homeopathic medicines have an antifungal activity in its attenuation potency. *Silicea* in 12C (13.5 ± 3.5) mm, *Mercurius solubilis* in 6C (12.5 ± 5.5) mm, 12C (15 ± 2.0) mm and 1M (11.5 ± 1.5) mm; *Lachesis* in 6C (15 ± 4.5) mm, 30C (17.5 ± 0.5) mm, 200C (15 ± 3.0) mm and 1M (16 ± 4.0) mm potencies when compared with vehicle control (8.5 ± 0.5) mm and positive control(0) mm showed maximum zone of inhibition as compared to *Silicea* 6C (8 ± 0) mm, 200C (9 ± 1.0) mm, 1M (8.5 ± 0.5) mm and *Mercurius solubilis* 30C (8 ± 1.0) mm.

5. CONCLUSION

This study demonstrates that homeopathic medicines *Silicea* in 12C, *Mercurius solubilis* in 6C, 12C and 1M; *Lachesis* in 6C, 30C, 200C and 1M had definite inhibitory action against *F.moniliforme* as compared to Bavastin, Terbinafine and ethanol 90% and can be used as an effective antifungal drugs. Further detailed analysis of various different potencies of homeopathic medicines is a subject testing on the principles of homeopathy *viz.*, "Similia Similibus Curentur" in identifying cellular level and exact molecular targets and mechanism of action of these ultra-high diluted medicines.

6. CONFLICT OF INTEREST

The author bears no conflict of interest in the entire study and there was no source of funding for the study.

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